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A STUDY OF BACTERIA IN ICE CREAM DURING STORAGE

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H. B. ELLENBERGER

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A STUDY OF BACTERIA IN ICE CREAM DURING STORAGE

A STUDY OF BACTERIA IN ICE CREAM DURING STORAGE

H. B. Ellenberger

Within very recent years ice cream has passed from a luxury, only occasionally indulged in, to one of the commonest of desserts and confections. The annual consumption of ice cream in the United States amounts to almost two gallons per capita, and consumers last year paid nearly three hundred million dollars to indulge their appetite for this popular dish.

Because of the size and the rapid growth of the industry, ice cream has drawn the attention of the food expert and the health officer. Other dairy products are forced to conform to certain arbitrary standards of composition and bacteria content, and ice cream is being given a great deal of attention in this respect. A few cities have already passed bacterial standards, and many more are studying the situation and would act if they were sure they could make an equitable ruling. To add to the knowledge of the subject, and in the hope of obtaining information applicable to the industry, certain studies have been undertaken by the writer to determine the influence of some of the more important factors on the bacteria content of ice cream, and particularly the effect of storage on the total number and the classes of bacteria found. Certain practices of plating, and the use of different media and different incubation temperatures, were studied in their relation to the bacterial analysis of ice cream.

PLAN OF WORK

The various experimental batches of ice cream were frozen in a ten-gallon brine freezer. Enough cream, direct from the separator, was obtained at one time to make up five ten-gallon mixes. The milk from which this cream was separated was received at one of the college skimming stations, about fifteen miles away, cooled, shipped in to the College on the following day, and separated. Bulk condensed milk, shipped direct from a condensery, was used in the groups of samples designated A and B, while those designated C and D contained only cream, sugar, vanilla, and gelatin.

All utensils used, except the freezer, were sterilized with live steam for several hours shortly before using. The freezer was steamed thoroly from a steam hose just before the various batches were frozen. Checks on its sterility were obtained by passing one-half liter of sterile water thru the freezer while it was in motion, and then plating samples of this water.

As soon as it was received from the separator, the cream was cooled, standardized, and divided into five batches, as follows:

No. 1 was mixed and frozen at once.

No. 2 was held for one day, and was then mixed and frozen. No platings were made from this batch. It was frozen in order to determine the influence of aging the cream on the swell.

No. 3 was held for two days, and was then mixed and frozen.

No. 4 was pasteurized at 145° F. for thirty minutes, cooled, held for two days, and then mixed and frozen.

No. 5 was mixed ready for freezing, except for the gelatin and the flavoring, pasteurized at 145° F. for thirty minutes, cooled, and held for two days, after which the gelatin and the flavoring were added and the mixture was frozen.

The temperature at which the batches were held was maintained at near 32° F.

After freezing, one packing can of ice cream from each batch was placed in an artificially cooled hardening room, where it was kept frozen at a temperature of from 10° to 15° F. Thus it was easily accessible for sampling. Samples for plating were taken from each of the ingredients entering the mix, from the mix itself, and from the freshly frozen ice cream. After hardening, the ice cream in each can was sampled and plated on the second, fourth, sixth, eighth, eleventh, fourteenth, seventeenth, twenty-first, twenty-fifth, twenty-ninth, forty-fifth, sixtieth, seventy-fifth, ninetieth, and one hundred and twentieth days.

Platings were made on lactose agar and on litmus lactose gelatin, by which the colonies were divided into acid-forming, inert, and liquefying groups. To further differentiate between the different groups of bacteria according to their action on milk, colonies were picked from agar plates and inoculated into tubes of sterile litmus milk. These milk tubes were then incubated at 37° C. for ten days, and the reaction produced in the milk was recorded on the second, fifth, and tenth days, thereby dividing the bacteria into groups of acid-coagulating, acid-forming, inert, alkali-forming, and peptonizing.

All plates were incubated at 20° C. and counted on the seventh day, except in the case of certain gelatin plates containing rapid liquefiers which had to be counted in a shorter time.

The gelatin was made up as follows: gelatin 15 per cent, peptone 1 per cent, beef extract 0.5 per cent, lactose 1 per cent. It was titrated to an acidity of +10, Fuller's scale.

The composition of the lactose agar used was: thread agar 1.5 per cent, peptone 1 per cent, beef extract 0.5 per cent, lactose 1 per cent. This was titrated to +10 acidity, Fuller's scale.

METHODS OF SAMPLING FOR BACTERIA TESTS

Thruout these studies, all samples for plating were taken in a uniform manner by first removing the surface of the ice cream to a depth of one-half inch with a sterile spoon, and then removing a sample with a sterile butter trier. The sample was transferred to an ordinary dilution bottle and allowed to melt at room temperature, after which it was thoroly mixed by shaking, and about one cubic centimeter was transferred with a sterile pipette to a weighing bottle and weighed. Dilutions were all calculated from the weight; therefore the counts of bacteria are expressed in the number per gram, not in the number per cubic centimeter.

SOURCES OF BACTERIA IN ICE CREAM

Since the larger part of the ice-cream mix is composed of cream or of milk and cream, it is natural to suppose that the bacteria content of freshly frozen ice cream is largely determined by the number of bacteria present in these materials. This is substantiated by Hammer (1912)[1] and by Bahlman (1914). It is of interest to note the numbers of bacteria present in each of the ingredients as they entered the various mixes thruout these trials, which were as follows:

	Minimum number of bacteria	Maximum number of bacteria
Standardized cream	1,150	37,600,000
Condensed milk	31,500	59,800,000
Sugar	20	255
Gelatin	48	891
Flavoring	10	321

---

[1] Dates in parenthesis refer to Bibliography, page 362.

In order to make sure that the freezer did not contaminate the ice cream, 500 cubic centimeters of sterile water was run thru it, as previously noted. Samples of this water when plated gave counts varying from 10 to 55 bacteria per cubic centimeter, with an average of 28.

From these figures it is readily seen that the cream and the condensed milk supplied most of the bacteria which entered the ice cream.

EFFECT OF FREEZING PROCESS ON NUMBER OF BACTERIA

In spite of the fact that, as has been shown, the number of bacteria in the freezer was negligible as a factor in increasing the bacteria count of the ice cream, yet in almost every case the fresh ice cream, as it came from the freezer, gave much higher counts than did the mix as it entered the freezer. An average increase of 48 per cent is shown in table 1 (page 336). Gordon (Gordon, Prescott, Heinemann, and Pease, 1914) obtained similar results. This may perhaps be accounted for in the breaking-up of clumps of bacteria by the beating received from the dasher during freezing. It is possible that thru freezing these clumps are rendered brittle, which would cause them to break apart more readily.

TOTAL NUMBERS OF BACTERIA IN ICE CREAM DURING STORAGE

Stiles (Stiles and Pennington, 1909:263–265) made counts at various intervals of the total numbers of bacteria in four samples of ice cream which he held frozen hard for a period of thirty-four days. These samples showed rather wide variations, but in general the number of bacteria increased up to the third day of storage, after which there was a gradual decrease to the fourteenth day. There was then a more rapid increase, until the highest counts were reached on the twenty-seventh day. On the thirty-fourth day the counts were about equal to those of the fourteenth day. These samples were purchased from retailers and their age at the time of purchase was not stated. Pennington (Stiles and Pennington, 1909:266–269) held eight samples of ice cream for periods of from three to ten days. The counts during these storage periods showed wide variations, but for the most part there was more or less decrease during the first twenty-four hours. The history of these samples before purchase was unknown.

Hammer (1912) stored twelve samples of ice cream for periods of from three to forty-four days. He concludes that the number of bacteria developing on agar at 37° C. does not increase during storage if the product is kept suitably hardened. Esten and Mason (1915) concluded, after holding several samples of ice cream for about a month, that "when ice cream is kept frozen for periods of at least a month there is no marked increase or decrease in the bacterial content as shown by litmus lactose gelatin plate cultures. The percentage of acid bacteria and of liquefying bacteria also remained fairly constant."

The average counts of the fourteen samples in the trials described in this paper, made at various times during the storage period of one hundred and twenty days and reported in table 1, indicate a slight decrease in total numbers during the first four days of storage, with a noticeable increase from the fourth to the eighth day, after which there is a gradual decline until the end of the period.

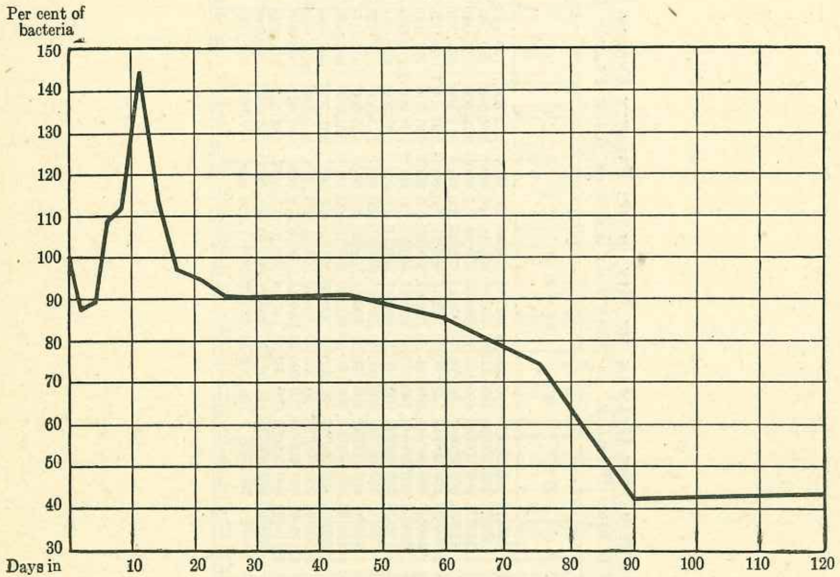

FIG. 75. AVERAGE PERCENTAGE INCREASE AND DECREASE OF BACTERIA IN FOURTEEN SAMPLES OF ICE CREAM DURING A STORAGE PERIOD OF 120 DAYS

The percentage increase or decrease in numbers of bacteria in each sample during the storage period, and also the average percentage variation on all samples, are shown in table 2. The latter is also charted by curve in figure 75. All percentages are calculated from the count of the freshly frozen ice cream. This shows a drop to 89.1 per cent of the initial

TABLE 1. Total Numbers of Bacteria per Gram in the Mix, in the Freshly Frozen Ice Cream, and in the Ice Cream at Various Periods during Storage, as Shown by Lactose Agar Plates Incubated at 20° C.

Sample	A 1	A 3	A 4	A 5	B 1	B 3	B 4	B 5	C 3	C 4	C 5	D 3	D 4	D 5	Average
Mix	7,065,000	10,500,000	5,990,000	500	18,240,000	25,850,000	39,900	25,200	20,400,000	107,600	176,400	3,700,000	6,900	7,950	6,579,246
Frozen	6,600,000	11,900,000	6,380,000	1,500	25,280,000	50,400,000	88,100	45,000	31,100,000	873,200	499,200	3,100,000	26,000	21,500	9,736,750
2d day	6,830,000	9,350,000	7,104,000	1,250	25,600,000	30,400,000	29,650	39,000	50,732,000	432,000	320,000	3,400,000	16,600	25,500	9,591,428
4th day	7,720,000	13,400,000	6,780,000	1,150	28,900,000	43,900,000	80,000	42,850	26,200,000	375,500	365,700	3,000,000	16,100	18,900	9,342,871
6th day	8,550,000	11,200,000	6,300,000	2,100	37,600,000	47,500,000	56,500	48,000	22,266,600	1,050,000	1,002,000	3,550,000	18,800	15,750	9,939,982
8th day	7,650,000	16,333,000	8,050,000	1,800	42,000,000	44,800,000	56,000	37,000	25,600,000	995,000	995,000	3,550,000	17,900	18,200	10,721,707
11th day	9,500,000	14,800,000	8,250,000	2,600	28,000,000	49,100,000	48,500	32,350	27,200,000	2,700,000	2,270,000	3,150,000	18,600	19,850	10,363,707
14th day	8,150,000	13,000,000	8,600,000	3,150	33,600,000	37,000,000	50,000	47,500	31,500,000	1,020,000	830,000	3,300,000	19,300	17,500	9,795,532
17th day	7,350,000	13,250,000	8,400,000	1,750	31,800,000	43,500,000	49,000	44,000	21,600,000	775,000	736,660	2,033,300	13,840	21,000	9,255,325
21st day	8,700,000	12,800,000	6,900,000	1,850	31,600,000	44,400,000	51,500	54,500	26,000,000	570,000	565,000	1,975,000	16,000	16,050	9,546,421
25th day	7,300,000	12,300,000	6,250,000	1,400	52,600,000	33,000,000	58,000	39,500	18,000,000	673,300	652,500	1,575,000	15,450	13,350	9,462,750
29th day	7,600,000	13,500,000	7,750,000	1,650	38,500,000	34,000,000	46,000	40,000	23,200,000	363,300	446,600	3,187,500	13,300	18,400	9,190,482
45th day	2,800,000	12,200,000	6,500,000	2,500	25,000,000	36,750,000	47,300	40,500	23,650,000	857,500	830,000	2,475,000	12,500	16,600	7,941,564
60th day	4,700,000	9,800,000	6,600,000	1,175	32,400,000	28,750,000	48,750	41,250	16,800,000	695,000	960,000	2,150,000	16,660	14,850	7,355,549
75th day	4,625,000	10,850,000	6,500,000	1,210	22,600,000	31,500,000	53,250	36,750	10,400,000	696,600	813,300	1,112,500	11,100	10,500	6,372,158
90th day	1,800,000	1,500,000	5,550,000	633	15,866,600	25,333,300	30,500	32,000	10,000,000	65,000	44,000	1,593,750	14,650	11,866	4,417,307
120th day	2,737,500	5,525,000	5,875,000	490	7,200,000	9,700,000	32,625	22,500	7,966,600	172,500	259,000	1,810,000	14,600	11,270	2,951,935

TABLE 2. Variation in Bacterial Counts, Expressed in Percentage of the Count of the Freshly Frozen Ice Cream

Sample	A 1	A 3	A 4	A 5	B 1	B 3	B 4	B 5	C 3	C 4	C 5	D 3	D 4	D 5	Average
Frozen	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2d day	103.5	78.6	111.3	83.3	101.3	60.3	33.6	86.7	163.1	49.5	64.1	109.7	63.8	118.6	87.7
4th day	117.0	112.6	106.3	76.7	114.3	87.1	90.8	95.2	84.2	43.0	73.2	96.8	61.9	87.9	89.1
6th day	129.5	94.1	98.7	140.0	148.7	94.2	64.1	106.7	71.6	120.2	200.7	114.5	72.3	73.2	109.2
8th day	115.9	137.2	126.2	120.0	166.1	88.9	63.6	82.2	82.3	113.9	199.3	114.5	68.8	84.6	111.7
11th day	143.9	124.4	129.3	173.3	110.7	97.4	55.0	71.9	87.4	309.2	454.7	101.6	71.5	92.3	144.5
14th day	123.5	109.2	134.8	210.0	132.9	73.4	56.7	105.5	101.3	116.8	166.3	106.4	74.2	81.4	113.7
17th day	111.4	111.3	131.7	116.7	125.8	86.3	55.6	97.8	69.4	88.7	147.6	65.6	53.2	97.7	97.1
21st day	131.8	107.6	108.1	123.3	125.0	88.1	58.4	121.1	83.6	65.3	113.2	63.7	61.5	74.6	94.7
25th day	110.6	103.4	98.0	93.3	208.1	65.5	65.8	87.8	57.9	77.1	130.7	50.8	59.4	62.1	90.8
29th day	115.1	113.4	121.5	110.0	152.3	67.5	52.2	88.9	74.6	41.6	89.5	102.8	51.1	85.6	90.4
45th day	42.4	102.5	101.9	166.7	98.9	72.9	53.7	90.0	76.0	98.2	166.3	79.8	48.1	77.2	91.0
60th day	71.2	82.3	103.4	78.3	128.2	57.0	55.3	91.7	54.0	79.6	192.3	69.3	64.1	69.1	85.4
75th day	70.1	91.2	101.9	80.7	89.4	62.5	60.4	81.7	33.4	79.8	162.9	35.9	42.7	48.8	74.4
90th day	27.3	12.6	87.0	42.2	62.8	50.3	34.6	71.1	32.1	7.4	8.8	51.4	56.3	55.2	42.8
120th day	41.5	46.4	92.1	32.7	28.5	19.2	37.0	50.0	25.6	19.7	51.9	58.4	56.1	52.4	43.7

count during the first four days of storage, after which there is a rapid increase to 144.5 per cent reached on the eleventh day, then just as rapid a drop back to 97.1 per cent on the seventeenth day, followed by a less noticeable gradual decrease to about 43 per cent at the ninetieth- and one-hundred-and-twentieth-day periods.

GROUPS OF BACTERIA FOUND DURING STORAGE

Groups shown by litmus gelatin plates

For a quick method of dividing the bacteria into groups, the litmus lactose gelatin plates were used. From these plates the colonies developing can be divided into three groups — acid-forming, inert, and liquefying. This method of grouping was used on samples of each mix and on the ice cream samples, which were plated on the eighth, seventeenth, twenty-ninth, forty-fifth, sixtieth, seventy-fifth, and ninetieth days of storage. The results obtained from each of the fourteen samples, expressed in percentage of the total count, are given in table 3. The average of these one hundred and five trials shows that 80.2 per cent of the total number of bacteria were acid formers, 14.9 per cent were inert, and 4.9 per cent were liquefiers. The acid-forming group shows a tendency to increase from the mix to the twenty-ninth day of storage, while the inert group shows a corresponding decrease. After the twenty-ninth day the acid formers show a decrease, while both the inert and the liquefying group show some increase.

A comparison of the groups developing from ice cream made of raw cream, with those in ice cream made of pasteurized cream, is shown in table 4. Sample 3 in each of the four trials was made from raw cream, while samples 4 and 5 were made from the same cream pasteurized. The pasteurized samples show a lower proportion of acid-forming bacteria thruout, but a higher proportion of the inert and liquefying groups.

Groups shown by litmus milk tubes

While nearly all of the acid-forming groups found on litmus gelatin plates produce acid in milk, the action on milk of the other two classes of bacteria, inert and liquefying, is very doubtful. Even among the acid-forming groups there are found subgroups of organisms showing very different action on milk; some of these cause coagulation, others do not,

TABLE 3. PERCENTAGES OF BACTERIA IN VARIOUS GROUPS AS SHOWN BY LITMUS LACTOSE GELATIN PLATES

Sample	A 1			A 3			A 4			A 5			B 1			B 3			B 4			B 5		
	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying
Mix	0.8	85.3	13.9	87.5	6.3	6.2	66.1	30.3	3.6	.....	.....	.....	85.3	13.2	1.5	87.9	9.1	3.0	64.8	31.0	4.2	70.0	20.0	10.0
8th day	80.0	16.0	4.0	79.5	11.7	8.8	90.0	10.0	0.0	77.7	22.3	0.0	96.2	1.3	2.5	90.0	3.4	6.6	55.0	45.0	0.0	85.7	14.3	0.0
17th day	50.0	25.0	25.0	100.0	0.0	0.0	70.0	20.0	10.0	93.3	2.8	3.9	93.6	6.4	0.0	100.0	0.0	0.0	80.0	20.0	0.0	100.0	0.0	0.0
29th day	76.4	11.9	11.7	89.4	8.0	2.6	81.8	18.2	0.0	94.6	2.4	3.0	98.0	1.0	1.0	91.7	0.0	8.3	92.1	6.8	1.1	93.1	6.9	0.0
45th day	87.5	0.0	12.5	76.9	23.1	0.0	82.4	17.6	0.0	62.5	12.5	25.0	92.9	7.1	0.0	82.7	13.9	3.4	75.0	18.8	6.2	76.9	8.0	15.1
60th day	91.6	4.3	4.1	95.5	0.0	4.5	60.0	13.3	26.7	80.3	16.7	3.0	82.1	17.9	0.0	98.3	1.7	0.0	95.4	4.6	0.0	83.3	16.7	0.0
75th day	87.5	12.5	0.0	80.0	15.0	5.0	88.8	11.2	0.0	60.7	37.7	1.6	96.2	2.6	1.2	92.0	6.0	2.0	88.8	11.2	0.0	77.0	19.2	3.8
90th day	81.2	12.6	6.2	82.6	17.4	0.0	90.9	0.0	9.1	67.5	25.7	6.8	84.1	14.3	1.6	79.2	12.5	8.3	78.1	12.8	9.1	69.2	19.3	11.5
Average	69.4	20.9	9.7	86.4	10.2	3.4	78.7	15.1	6.2	76.7	17.1	6.2	91.0	8.0	1.0	90.2	5.8	4.0	78.6	18.8	2.6	81.9	13.1	5.0

Sample	C 3			C 4			C 5			D 3			D 4			D 5			Average of all samples		
	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefyin
Mix	97.6	0.0	2.4	67.3	30.7	2.0	85.8	14.2	0.0	79.0	9.4	11.6	75.0	12.5	12.5	80.0	15.0	5.0	72.9	21.3	5.8
8th day	89.7	7.8	2.5	85.6	14.4	0.0	78.2	21.8	0.0	92.3	5.1	2.6	82.5	17.5	0.0	63.6	36.4	0.0	81.9	16.2	1.9
17th day	.....	.....	.....	80.0	0.0	20.0	84.2	10.6	5.2	94.5	3.7	1.8	93.8	6.2	0.0	95.6	2.2	2.2	87.3	7.5	5.2
29th day	95.5	3.4	1.1	80.0	20.0	0.0	85.0	15.0	0.0	97.1	1.0	1.9	95.1	4.9	0.0	93.9	6.1	0.0	90.3	7.5	2.2
45th day	.....	.....	.....	90.4	9.6	0.0	80.0	20.0	0.0	94.8	3.9	1.3	97.4	1.7	0.9	4.9	91.5	3.6	77.3	17.5	5.2
60th day	90.0	9.2	0.8	66.7	33.3	0.0	60.0	40.0	0.0	92.0	8.0	0.0	65.6	34.4	0.0	81.4	18.6	0.0	81.6	15.6	2.8
75th day	73.0	21.6	5.4	66.7	0.0	33.3	40.0	30.0	30.0	72.2	22.3	5.5	.....	.....	.....	45.6	26.4	28.0	74.5	16.6	8.9
90th day	71.0	18.8	10.2	59.1	27.3	13.6	.....	.....	.....	53.9	30.7	15.4	.....	.....	.....	.....	.....	.....	74.3	17.4	8.3
Average	86.1	10.1	3.8	74.5	16.9	8.6	73.3	21.7	5.0	84.5	10.5	5.0	84.9	12.9	2.2	66.4	28.0	5.6	80.2	14.9	4.9

TABLE 4. PERCENTAGES OF BACTERIA IN VARIOUS GROUPS AS SHOWN ON LITMUS LACTOSE GELATIN, FROM ICE CREAM MADE OF RAW AND OF PASTEURIZED CREAM

Sample	Raw cream			Pasteurized cream		
	Acid-forming	Inert	Liquefying	Acid-forming	Inert	Liquefying
A 3	86.4	10.2	3.4	....	....	....
A 4	....	....	....	78.7	15.1	6.2
A 5	....	....	....	76.7	17.1	6.2
B 3	90.2	5.8	4.0	....	....	....
B 4	....	....	....	78.6	18.8	2.6
B 5	....	....	....	81.9	13.1	5.0
C 3	86.1	10.1	3.8	....	....	....
C 4	....	....	....	74.5	16.9	8.6
C 5	....	....	....	73.3	21.7	5.0
D 3	84.5	10.5	5.0	....	....	....
D 4	....	....	....	84.9	12.9	2.2
D 5	....	....	....	66.4	28.0	5.6
Average	86.8	9.2	4.0	76.9	17.9	5.2

and still others ferment the milk sugar, forming gas. To divide the organisms into groups according to the action shown on milk, the milk-tube method, previously mentioned, was used. By this means Ayers and Johnson (1915) obtained the following average from 71 samples of commercial ice cream tested during the summer in Washington, D. C.:

	Per cent
Acid-coagulating	49.82
Acid-forming	20.72
Inert	13.98
Alkali-forming	1.86
Peptonizing	13.62

In the writer's experiments, groups were determined by the milk-tube method from the same samples as were used with the gelatin plates. Individual colonies were picked from a representative agar plate and inoculated into tubes of litmus milk, which were incubated at 37° C. On the basis of the ten-day reaction in this litmus milk, the bacteria were divided into five groups — acid-coagulating, acid-forming, inert, alkali-forming, and peptonizing. The results, expressed in percentages of the total count, are summarized in table 5. The average of these one hundred

TABLE 5. PERCENTAGES OF BACTERIA IN VARIOUS GROUPS AS SHOWN BY LITMUS MILK TUBES

Sample	A 1					A 3					A 4					A 5				
	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing
Mix	29.4	5.9	54.9	7.8	2.0	16.7	9.5	71.4	0	2.4	39.6	54.7	3.8	0	1.9	40.0	0.0	60.0	0	0.0
8th day	46.2	11.5	42.3	0.0	0.0	28.6	14.3	57.1	0	0.0	23.3	2.1	72.5	0	2.1	48.0	24.0	24.0	0	4.0
17th day	48.7	10.2	38.5	0.0	2.6	44.9	6.1	49.0	0	0.0	27.9	9.3	62.8	0	0.0	78.6	14.3	7.1	0	0.0
29th day	51.3	5.1	43.6	0.0	0.0	22.4	4.1	73.5	0	0.0	26.1	0.0	73.9	0	0.0	62.9	34.3	2.8	0	0.0
45th day	40.0	4.0	56.0	0.0	0.0	32.4	14.7	52.9	0	0.0	29.0	6.5	64.5	0	0.0	78.3	13.0	8.7	0	0.0
60th day	30.0	5.0	65.0	0.0	0.0	31.1	11.1	57.8	0	0.0	7.8	5.9	86.3	0	0.0	83.8	5.4	5.4	0	5.4
75th day	41.0	7.7	51.3	0.0	0.0	29.0	10.5	60.5	0	0.0	23.1	2.6	71.8	0	2.5	57.1	20.0	20.0	0	2.9
90th day	65.2	4.4	30.4	0.0	0.0	68.8	31.2	0.0	0	0.0	22.8	8.6	68.6	0	0.0	60.9	30.4	4.4	0	4.3
Average	44.0	6.7	47.7	1.0	0.6	34.2	12.7	52.8	0	0.3	25.0	11.2	63.0	0	0.8	63.7	17.7	16.5	0	2.1

Sample	B 1					B 3					B 4					B 5				
	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing
Mix	51.9	25.0	19.3	1.9	1.9	45.5	22.7	27.3	4.5	0.0	51.1	9.0	6.6	31.1	2.2	71.4	23.8	0.0	0	4.8
8th day	65.1	27.9	7.0	0.0	0.0	43.4	15.1	41.5	0.0	0.0	66.7	26.2	4.7	2.4	0.0	69.7	15.2	12.1	0	3.0
17th day	38.2	20.6	41.2	0.0	0.0	58.6	13.8	24.1	0.0	3.5	64.0	16.0	16.0	4.0	0.0	50.0	15.4	26.9	0	7.7
29th day	50.0	27.3	20.5	2.2	0.0	29.4	38.2	32.4	0.0	0.0	52.5	32.5	15.0	0.0	0.0	61.3	25.8	12.9	0	0.0
45th day	21.6	40.6	37.8	0.0	0.0	47.3	23.7	29.0	0.0	0.0	26.7	53.3	20.0	0.0	0.0	81.4	11.6	4.7	0	2.3
60th day	40.4	27.7	31.9	0.0	0.0	26.7	40.0	31.1	2.2	0.0	72.0	26.0	2.0	0.0	0.0	85.7	12.5	0.0	0	1.8
75th day	40.6	27.0	32.4	0.0	0.0	53.2	14.9	31.9	0.0	0.0	53.8	35.9	10.3	0.0	0.0	53.7	43.9	2.4	0	0.0
90th day	17.0	76.0	7.0	0.0	0.0	50.0	46.3	1.9	0.0	1.8	89.3	10.7	0.0	0.0	0.0	70.0	30.0	0.0	0	0.0
Average	40.6	34.0	24.7	0.5	0.2	44.3	26.8	27.4	0.8	0.7	59.5	26.2	9.3	4.7	0.3	67.9	22.3	7.4	0	2.4

TABLE 5 (*concluded*)

Sample	C 3					C 4					C 5				
	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing
Mix	61.0	4.9	31.7	0	2.4	16.7	77.8	5.5	0	0.0	6.3	87.5	6.2	0.0	0.0
8th day	38.9	38.9	22.2	0	0.0	24.0	68.0	8.0	0	0.0	14.8	81.5	3.7	0.0	0.0
17th day	41.3	41.3	17.4	0	0.0	3.2	93.6	0.0	0	3.2	11.5	61.6	23.1	0.0	3.8
29th day	40.5	50.0	7.1	0	2.4	41.4	55.2	3.4	0	0.0	19.2	80.8	0.0	0.0	0.0
45th day	48.9	39.5	11.6	0	0.0	....	....	....	....	....	34.0	66.0	0.0	0.0	0.0
60th day	67.7	25.8	6.5	0	0.0	3.6	89.2	3.6	0	3.6	12.9	80.7	3.2	0.0	3.2
75th day	56.3	29.1	14.6	0	0.0	21.9	78.1	0.0	0	0.0	10.7	89.3	0.0	0.0	0.0
90th day	74.1	18.5	7.4	0	0.0	56.3	37.5	0.0	0	6.2	36.4	45.4	0.0	9.1	9.1
Average	53.6	31.0	14.8	0	0.6	23.9	71.3	2.9	0	1.9	18.3	74.1	4.5	1.1	2.0

Sample	D 3					D 4					D 5					Average of all samples				
	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing
Mix	34.8	52.2	4.3	0	8.7	56.0	40.0	4.0	0	0.0	56.4	35.9	5.1	0	2.6	41.2	32.1	21.4	3.2	2.1
8th day	36.7	36.7	23.3	0	3.3	95.8	2.1	0.0	0	2.1	55.3	31.6	13.1	0	0.0	46.9	28.2	23.7	0.2	1.0
17th day	60.9	13.0	26.1	0	0.0	87.9	3.0	9.1	0	0.0	81.2	14.6	4.2	0	0.0	49.8	23.7	24.7	0.3	1.5
29th day	27.0	24.3	43.3	0	5.4	57.8	35.5	6.7	0	0.0	53.3	31.1	15.6	0	0.0	42.5	31.7	25.0	0.2	0.6
45th day	38.3	23.5	38.2	0	0.0	26.5	28.6	42.9	0	2.0	50.8	34.4	13.1	0	1.7	42.7	27.6	29.2	0.0	0.5
60th day	38.5	26.9	30.8	0	3.8	94.6	5.4	0.0	0	0.0	97.8	2.2	0.0	0	0.0	49.5	26.0	23.1	0.1	1.3
75th day	41.4	27.6	20.7	0	10.3	92.4	3.8	0.0	0	3.8	86.4	13.6	0.0	0	0.0	47.2	28.8	22.6	0.0	1.4
90th day	56.5	8.7	34.8	0	0.0	5.2	94.8	0.0	0	0.0	0.0	80.0	15.0	0	5.0	48.0	37.3	12.1	0.7	1.9
Average	41.8	26.6	27.7	0	3.9	64.5	26.7	7.8	0	1.0	60.1	30.4	8.3	0	1.2	46.0	29.4	22.7	0.6	1.3

and eleven sets shows a total acid group of 75.4 per cent. Of the total number, 46 per cent belong to the acid-coagulating group, 29.4 per cent to the acid-forming group, 22.7 per cent to the inert group, 0.6 per cent to the alkali-forming group, and 1.3 per cent to the peptonizing group.

The averages of the fourteen samples at the different periods show no uniform tendency to increase or to decrease during storage. This fact, considered in connection with the total counts at these different periods as shown in table 1, indicates that there may be a slow growth of the various groups of organisms during the first week or ten days of storage and that after that time the bacteria present merely live in an inactive state, with a gradual dying-off of all groups. The breaking apart of clumps of bacteria is another possible explanation of the increase in count during the first days of storage. Whether or not this is probable was not determined.

The difference in percentages of bacteria in the groups from the ice cream made of raw cream and in those from the ice cream made of pasteurized cream is shown in table 6. The most noticeable difference is that in all except one sample the pasteurized-cream group shows but few inert bacteria as compared to the raw-cream group. There is at the same time an increase in the total acid group, in some cases appearing in the acid-coagulating group and in other cases in the acid-forming group.

TABLE 6. PERCENTAGES OF BACTERIA IN VARIOUS GROUPS AS SHOWN BY LITMUS MILK TUBES, FROM ICE CREAM MADE OF RAW AND OF PASTEURIZED CREAM

Sample	Raw cream					Pasteurized cream				
	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing	Acid-coagulating	Acid-forming	Inert	Alkali-forming	Peptonizing
A 3	34.2	12.7	52.8	0.0	0.3	......	......	....	......	......
A 4	......	......	....	......	......	25.0	11.2	63.0	0.0	0.8
A 5	......	......	....	......	......	63.7	17.7	16.5	0.0	2.1
B 3	44.3	26.8	27.4	0.8	0.7	......	......	....	......	......
B 4	......	......	....	......	......	59.5	26.2	9.3	4.7	0.3
B 5	......	......	....	......	......	67.9	22.3	7.4	0.0	2.4
C 3	53.6	31.0	14.8	0.0	0.6	......	......	....	......	......
C 4	......	......	....	......	......	23.9	71.3	2.9	0.0	1.9
C 5	......	......	....	......	......	18.3	74.1	4.5	1.1	2.0
D 3	41.8	26.6	27.7	0.0	3.9	......	......	....	......	......
D 4	......	......	....	......	......	64.5	26.7	7.8	0.0	1.0
D 5	......	......	....	......	......	60.1	30.4	8.3	0.0	1.2
Average	43.4	24.3	30.7	0.2	1.4	47.9	35.0	14.9	0.7	1.5

TABLE 7. NUMBERS OF BACTERIA OF BACILLUS COLI GROUP AS SHOWN BY ENDO'S MEDIUM

(In bacteria per gram)

Sample	A 1	A 3	A 4	A 5	B 1	B 3	B 4	B 5	C 3	C 4	C 5	D 3	D 4	D 5
Mix	.....	.....	.....	.....	.....	.....	.....	.....	2,400	0	0	720	25	0
8th day	.....	.....	.....	.....	.....	.....	.....	.....	2,500	5	0	715	20	0
17th day	.....	.....	.....	.....	.....	.....	.....	.....	450	0	0	350	5	0
29th day	200	200	1,000	0	8,000	10,000	0	0	0	0	0	0	0	0
45th day	250	150	535	0	6,250	8,000	0	0	0	0	0	215	0	0
60th day	0	0	115	0	310	280	0	0	0	0	0	0	0	0
75th day	0	30	0	0	40	250	0	0	0	0	0	75	0	15
90th day	260	10	55	0	40	200	0	0	0	0	0	0	0	0

The Bacillus coli group

Since the presence in any considerable number of members of the *Bacillus coli* group in dairy products is looked upon with suspicion, platings were made at various periods on Endo's medium, to determine whether or not organisms of this class would develop in ice cream during storage. The numbers found in the different samples at various intervals are given in table 7. This shows no increase, but on the contrary a decided falling off, in numbers of bacteria during storage.

Groups as shown by litmus gelatin and by litmus milk compared

In order to compare in a general way the groups of bacteria as shown by litmus gelatin plates and by litmus milk tubes, the five groups from the milk tubes were reduced to three by combining the two acid-forming groups and including the alkali-forming with the inert group. The liquefiers of gelatin are here compared to the peptonizers of milk. The comparisons are shown in tables 8 to 16, inclusive, table 16 being a summary of the eight tables preceding.

TABLE 8. COMPARISON OF THE MIXES

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	0.8	35.3	85.3	62.7	13.9	2.0
A 3	87.5	26.2	6.3	71.4	6.2	2.4
A 4	66.1	94.3	30.3	3.8	3.6	1.9
A 5	....	40.0	....	60.0	....	0.0
B 1	85.3	76.9	13.2	21.2	1.5	1.9
B 3	87.9	68.2	9.1	31.8	3.0	0.0
B 4	64.8	60.1	31.0	37.7	4.2	2.2
B 5	70.0	95.2	20.0	0.0	10.0	4.8
C 3	97.6	65.9	0.0	31.7	2.4	2.4
C 4	67.3	94.5	30.7	5.5	2.0	0.0
C 5	85.8	93.8	14.2	6.2	0.0	0.0
D 3	79.0	87.0	9.4	4.3	11.6	8.7
D 4	75.0	96.0	12.5	4.0	12.5	0.0
D 5	80.0	92.3	15.0	5.1	5.0	2.6
Average	72.9	73.3	21.3	24.6	5.8	2.1

TABLE 9. COMPARISON OF THE EIGHTH-DAY SAMPLES

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	80.0	57.7	16.0	42.3	4.0	0.0
A 3	79.5	42.9	11.7	57.1	8.8	0.0
A 4	90.0	25.4	10.0	72.5	0.0	2.1
A 5	77.7	72.0	22.3	24.0	0.0	4.0
B 1	96.2	93.0	1.3	7.0	2.5	0.0
B 3	90.0	58.5	3.4	41.5	6.6	0.0
B 4	55.0	92.9	45.0	7.1	0.0	0.0
B 5	85.7	84.9	14.3	12.1	0.0	3.0
C 3	89.7	77.8	7.8	22.2	2.5	0.0
C 4	85.6	92.0	14.4	8.0	0.0	0.0
C 5	78.2	96.3	21.8	3.7	0.0	0.0
D 3	92.3	73.4	5.1	23.3	2.6	3.3
D 4	82.5	97.9	17.5	0.0	0.0	2.1
D 5	63.6	86.9	36.4	13.1	0.0	0.0
Average	81.9	75.1	16.2	23.9	1.9	1.0

TABLE 10. COMPARISON OF THE SEVENTEENTH-DAY SAMPLES

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	50.0	58.9	25.0	38.5	25.0	2.6
A 3	100.0	51.0	0.0	49.0	0.0	0.0
A 4	70.0	37.2	20.0	62.8	10.0	0.0
A 5	93.3	92.9	2.8	7.1	3.9	0.0
B 1	93.6	58.8	6.4	41.2	0.0	0.0
B 3	100.0	72.4	0.0	24.1	0.0	3.5
B 4	80.0	80.0	20.0	20.0	0.0	0.0
B 5	100.0	65.4	0.0	26.9	0.0	7.7
C 3	....	82.6	....	17.4	....	0.0
C 4	80.0	96.8	0.0	0.0	20.0	3.2
C 5	84.2	73.1	10.6	23.1	5.2	3.8
D 3	94.5	73.9	3.7	26.1	1.8	0.0
D 4	93.8	90.9	6.2	9.1	0.0	0.0
D 5	95.6	95.8	2.2	4.2	2.2	0.0
Average	87.3	73.5	7.5	25.0	5.2	1.5

TABLE 11. COMPARISON OF THE TWENTY-NINTH-DAY SAMPLES

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	76.4	56.4	11.9	43.6	11.7	0.0
A 3	89.4	26.5	8.0	73.5	2.6	0.0
A 4	81.8	26.1	18.2	73.9	0.0	0.0
A 5	94.6	97.2	2.4	2.8	3.0	0.0
B 1	98.0	77.3	1.0	22.7	1.0	0.0
B 3	91.7	67.6	0.0	32.4	8.3	0.0
B 4	92.1	85.0	6.8	15.0	1.1	0.0
B 5	93.1	87.1	6.9	12.9	0.0	0.0
C 3	95.5	90.5	3.4	7.1	1.1	2.4
C 4	80.0	96.6	20.0	3.4	0.0	0.0
C 5	85.0	100.0	15.0	0.0	0.0	0.0
D 3	97.1	51.3	1.0	43.3	1.9	5.4
D 4	95.1	93.3	4.9	6.7	0.0	0.0
D 5	93.9	84.4	6.1	15.6	0.0	0.0
Average	90.3	74.2	7.5	25.2	2.2	0.6

TABLE 12. COMPARISON OF THE FORTY-FIFTH-DAY SAMPLES

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	87.5	44.0	0.0	56.0	12.5	0.0
A 3	76.9	47.1	23.1	52.9	0.0	0.0
A 4	82.4	35.5	17.6	64.5	0.0	0.0
A 5	62.5	91.3	12.5	8.7	25.0	0.0
B 1	92.9	62.2	7.1	37.8	0.0	0.0
B 3	82.7	71.0	13.9	29.0	3.4	0.0
B 4	75.0	8.00	18.8	20.0	6.2	0.0
B 5	76.9	93.0	8.0	4.7	15.1	2.3
C 3	....	88.4	....	11.6	....	0.0
C 4	90.4	......	9.6	......	0.0	....
C 5	8.00	100.0	20.0	0.0	0.0	0.0
D 3	94.8	61.8	3.9	38.2	1.3	0.0
D 4	97.4	55.1	1.7	42.9	0.9	2.0
D 5	4.9	85.2	91.5	13.1	3.6	1.7
Average	77.3	70.3	17.5	29.2	5.2	0.5

TABLE 13. Comparison of the Sixtieth-Day Samples

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	91.6	35.0	4.3	65.0	4.1	0.0
A 3	95.5	42.2	0.0	57.8	4.5	0.0
A 4	60.0	13.7	13.3	86.3	26.7	0.0
A 5	80.3	89.2	16.7	5.4	3.0	5.4
B 1	82.1	68.1	17.9	31.9	0.0	0.0
B 3	98.3	66.7	1.7	33.3	0.0	0.0
B 4	95.4	98.0	4.6	2.0	0.0	0.0
B 5	83.3	98.2	16.7	0.0	0.0	1.8
C 3	90.0	93.5	9.2	6.5	0.8	0.0
C 4	66.7	92.8	33.3	3.6	0.0	3.6
C 5	60.0	93.6	40.0	3.2	0.0	3.2
D 3	92.0	65.4	8.0	30.8	0.0	3.8
D 4	65.6	100.0	34.4	0.0	0.0	0.0
D 5	81.4	100.0	18.6	0.0	0.0	0.0
Average	81.6	75.5	15.6	23.2	2.8	1.3

TABLE 14. Comparison of the Seventy-fifth-Day Samples

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	87.5	48.7	12.5	51.3	0.0	0.0
A 3	80.0	39.5	15.0	60.5	5.0	0.0
A 4	88.8	25.7	11.2	71.8	0.0	2.5
A 5	60.7	77.1	37.7	20.0	1.6	2.9
B 1	96.2	67.6	2.6	32.4	1.2	0.0
B 3	92.0	68.1	6.0	31.9	2.0	0.0
B 4	88.8	89.7	11.2	10.3	0.0	0.0
B 5	77.0	97.6	19.2	2.4	3.8	0.0
C 3	73.0	85.4	21.6	14.6	5.4	0.0
C 4	66.7	100.0	0.0	0.0	33.3	0.0
C 5	40.0	100.0	30.0	0.0	30.0	0.0
D 3	72.2	69.0	22.3	20.7	5.5	10.3
D 4	....	96.2	....	0.0	....	3.8
D 5	45.6	100.0	26.4	0.0	28.0	0.0
Average	74.5	76.0	16.6	22.6	8.9	1.4

TABLE 15. Comparison of the Ninetieth-Day Samples

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
A 1	81.2	69.6	12.6	30.4	6.2	0.0
A 3	82.6	100.0	17.4	0.0	0.0	0.0
A 4	90.9	31.4	0.0	68.6	9.1	0.0
A 5	67.5	91.3	25.7	4.4	6.8	4.3
B 1	84.1	93.0	14.3	7.0	1.6	0.0
B 3	79.2	96.3	12.5	1.9	8.3	1.8
B 4	78.1	100.0	12.8	0.0	9.1	0.0
B 5	69.2	100.0	19.3	0.0	11.5	0.0
C 3	71.0	92.6	18.8	7.4	10.2	0.0
C 4	59.1	93.8	27.3	0.0	13.6	6.2
C 5	....	81.8	....	9.1	....	9.1
D 3	53.9	65.2	30.7	34.8	15.4	0.0
D 4	....	100.0	....	0.0	....	0.0
D 5	....	80.0	....	15.0	....	5.0
Average	74.3	85.3	17.4	12.8	8.3	1.9

TABLE 16. Comparison of the Averages of Samples at Different Periods

Samples	Acid		Inert		Liquefiers	Peptonizers
	Gelatin	Milk	Gelatin	Milk	Gelatin	Milk
Mix	72.9	73.3	21.3	24.6	5.8	2.1
8th day	81.9	75.1	16.2	23.9	1.9	1.0
17th day	87.3	73.5	7.5	25.0	5.2	1.5
29th day	90.3	74.2	7.5	25.2	2.2	0.6
45th day	77.3	70.3	17.5	29.2	5.2	0.5
60th day	81.6	75.5	15.6	23.2	2.8	1.3
75th day	74.5	76.0	16.6	22.6	8.9	1.4
90th day	74.3	85.3	17.4	12.8	8.3	1.9

The gelatin plates show the higher proportion of acid formers in all periods except the mix, the seventy-fifth day, and the ninetieth day, and a higher proportion of liquefiers than of peptonizers from milk tubes at every period. Undoubtedly there were some colonies which liquefied gelatin but did not peptonize milk. With these two groups larger in the gelatin than the corresponding ones of the milk tubes, the inert group, of course, must be smaller. In only one case, on the ninetieth day, did the gelatin show as high a percentage in the inert group as did the milk tubes.

ORGANISMS SURVIVING ONE HUNDRED AND TWENTY DAYS OF STORAGE

For a more detailed study of the classes and types of bacteria which survived storage, twenty typical colonies were picked from a representative agar plate of each of the fourteen samples plated on the one hundred and twentieth day. These colonies were first transferred to fermentation tubes of lactose bouillon. From these tubes stained slides were made and examined under the microscope, and transfers were made to gelatin stabs, agar slants, and litmus milk tubes. All were incubated at 20° C., the agar plates for seven days, the lactose bouillon and agar slants for five days, and the gelatin stabs and litmus milk tubes for fifteen days. A record was kept of all daily changes during these periods.

TABLE 17. GROUPS AND TYPES OF ORGANISMS SURVIVING 120 DAYS OF STORAGE

Groups or types of organisms	Number from six raw samples	Number from eight pasteurized samples	Total number
Bacterium lactis acidi group			
Coagulated milk in 1 to 4 days	31	2	33
Coagulated milk in 5 to 10 days	7	3	10
Coagulated milk in 11 to 15 days	7	8	15
Milk not coagulated in 15 days	69	126	195
Appearing in long chains, acid formers but with no coagulation	2	8	10
Total	116	147	263
Bacteria, forming acid and liquefying gelatin	0	4	4
Cocci, forming acid, yellow growth on agar	0	2	2
Cocci, no action in milk	1	1	2
Diplococci, peptonizing milk and liquefying gelatin	1	0	1
Long rods, peptonizing milk and liquefying gelatin	1	3	4
Long rods, alkali producers	1	1	2
Streptothrix	0	1	1
Mold	0	1	1
Total miscellaneous	4	13	17

Of the 280 cultures 269 were acid producers, 263 of these apparently belonging to the *Bacterium lactis acidi* group. These varied from small rods which could scarcely be distinguished from cocci, to slightly larger distinct rods. Many formed short chains, but only ten appeared in long chains.

For comparison, a sample of good commercial starter was plated and colonies were examined and grown as described. Little difference could be noted between these acid-forming organisms and those isolated from the storage ice cream. In many cases they appeared to be identical.

The numbers of the various groups and types of organisms appearing among these 280 cultures are given in table 17.

INFLUENCE OF INCUBATION TEMPERATURES ON THE TOTAL COUNTS

Before starting to make bacteria counts of experimental freezings of ice cream, it was deemed best to determine the incubation temperature most favorable to the growth of such bacteria in plates. For this purpose lactose agar of the composition previously mentioned was chosen as the nutrient medium. Twenty-two samples of ice cream were procured from nine manufacturers, and were plated in three dilutions. Four sets of plates in duplicate were made from each dilution, and each set was incubated at a different temperature. The temperature and the time of incubation were as follows:

Two days at 37° C. on all samples;
Five days at 30° C. on all samples;
Seven days at 20° C. on all samples;
Thirty days at 0° C. and two days at 37° C. on samples C to P inclusive;
Five days at 20° C. and two days at 37° C. on samples Q to W inclusive.

The results are shown in table 18. It will be noted that the average count of these samples from plates incubated at 30° C. for five days was almost two and one-half times the count of the same samples from plates incubated at 37° C. for two days. In no case did the 37° plates show as high a count as the 30° plates. The plates incubated at 20° C. showed an average count three times as great as those incubated at 37° C. In this case the 20° plates gave a higher count than the 30° plates in seventeen cases, while the 30° plates gave higher results in only five cases.

TABLE 18. EFFECT OF DIFFERENT TEMPERATURES OF INCUBATION OF PLATES ON THE TOTAL COUNT OF BACTERIA IN ICE CREAM

Sample	Time and temperature of incubation									
	Two days at 37° C.	Per cent	Five days at 30° C.	Per cent	Seven days at 20° C.	Per cent	Thirty days at 0° C. and two days at 37° C.	Per cent	Five days at 20° C. and two days at 37° C.	Per cent
A	285,000	100	520,000	182.4	675,000	236.8	....	......	....	......
B	7,750,000	100	14,550,000	187.7	16,500,000	212.9	....	......	....	......
C	590,000	100	1,580,000	267.8	1,695,000	287.3	315,000	53.4	....	......
D	2,850,000	100	6,900,000	242.1	5,460,000	191.6	830,000	29.1	....	......
E	1,985,000	100	4,222,500	212.7	4,125,000	207.8	340,000	17.1	....	......
F	360,000	100	495,000	137.5	520,000	144.4	75,000	20.8	....	......
G	1,815,000	100	8,950,000	493.1	10,550,000	581.3	6,860,000	378.0	....	......
H	4,910,000	100	12,500,000	254.6	14,950,000	304.5	4,215,000	85.8	....	......
I	3,095,000	100	10,650,000	344.1	10,550,000	340.9	5,315,000	171.7	....	......
J	2,453,000	100	4,420,000	180.2	4,655,000	189.8	820,000	33.4	....	......
L	39,900,000	100	48,500,000	121.5	56,800,000	142.3	50,133,133	125.6	....	......
M	300,000	100	1,600,000	533.3	2,802,500	934.2	260,000	86.7	....	......
N	11,900,000	100	35,400,000	297.5	70,500,000	592.4	7,250,000	60.9	....	......
O	77,000,000	100	125,000,000	162.3	183,500,000	238.3	182,000,000	236.4	....	......
P	4,630,000	100	6,750,000	145.8	5,950,000	128.5	5,195,000	112.2	....	......
Q	10,300,000	100	27,250,000	264.6	26,233,333	254.7	....	......	27,100,000	263.1
R	2,245,000	100	3,703,333	164.9	5,750,000	256.1	....	......	5,750,000	256.1
S	580,000	100	995,000	171.5	1,340,000	231.0	....	......	1,410,000	243.1
T	8,273,333	100	38,750,000	468.4	47,775,000	577.4	....	......	39,466,666	477.0
U	12,250,000	100	22,833,333	186.4	34,850,000	284.5	....	......	33,700,000	275.1
V	1,780,000	100	3,782,500	212.5	4,953,333	278.3	....	......	4,393,333	246.8
W	106,500,000	100	160,500,000	150.7	198,500,000	186.4	....	......	157,000,000	147.4
Average of 22 samples	....	100	....	244.6	....	309.2	....	......	....	......
Average of 13 samples	....	100	....	261.0	....	329.5	....	108.5	....	......
Average of 7 samples	....	100	....	231.3	....	295.5	....	......	....	272.7

The 0°-37° plates from thirteen samples gave a lower count than the 20° plates in every case, and a lower count than the 37° plates in eight of the thirteen trials.

Comparison of the 20°-37° plates with the 20° plates seemed to show that there was no advantage in incubating at higher temperatures after removing the plates from the 20° incubator.

Because of these results it was decided to incubate the plates from the experimental samples of ice cream at 20° C. for seven days, this temperature tending to show more nearly the true number of bacteria present than any of the other temperatures used.

NUMBERS OF BACTERIA FOUND IN ICE-CREAM GELATIN PREPARED BY DIFFERENT METHODS

Three of the commonest methods of dissolving gelatin for use in ice cream are as follows: (1) soaking in cold water for about thirty minutes, then heating to from 125° to 130° F. until dissolved; (2) soaking in cold water for thirty minutes, then heating to from 160° to 170° F.; (3) pouring the gelatin into boiling water and stirring it until dissolved.

In order to determine the effect of these methods on the bacteria content, fifteen samples of gelatin were procured from various manufacturers and dealers and were prepared by each of the methods. Platings from each preparation were made on litmus lactose gelatin and incubated at 20° C. The results are shown in table 19. The average total count of the fifteen samples when dissolved at a temperature of from 125° to 130° F. was 388,162; when dissolved at from 160° to 170° F. the average was only 1172; and when dissolved in boiling water the count was further reduced to an average of 530. On the other hand, the proportion of liquefiers relative to the total count was in most cases increased with the higher temperature. Occasionally the jelly from gelatin dissolved in boiling water had a slightly disagreeable flavor, and if it was held at this temperature for a considerable time some of the jellying power seemed to be destroyed. It therefore seemed best to prepare the gelatin for the experimental batches of ice cream by the second method — soaking in cold water, and then heating to from 160° to 170° F. just before using.

TABLE 19. EFFECT OF DIFFERENT METHODS OF DISSOLVING GELATIN ON THE BACTERIA COUNT

(The numbers expressed are in terms of bacteria in one gram of dry gelatin)

Sample	Method 1 — dissolving at 125° to 130° F.			Method 2 — dissolving at 160° to 170° F.			Method 3 — dissolving in boiling water		
	Total count	Number of liquefiers	Per cent of liquefiers	Total count	Number of liquefiers	Per cent of liquefiers	Total count	Number of liquefiers	Per cent of liquefiers
A	430	310	72.1	70	5	7.1	24	4	16.7
B	2,396,000	6,000	0.3	380	265	69.7	82	64	78.0
C	118,500	3,500	2.9	760	385	50.7	26	10	38.5
D	845,000	13,500	1.6	5,700	5,700	100.0	3,200	3,200	100.0
E	55,500	7,000	12.6	5,720	5,720	100.0	3,060	3,060	100.0
G	91,000	0	0.0	215	130	60.5	105	60	57.1
H	19,000	1,000	5.3	200	100	50.0	5	0	0.0
I	21,000	1,000	4.8	485	190	39.2	60	0	0.0
J	54,000	7,000	13.0	1,030	1,030	100.0	540	540	100.0
K	159,000	9,000	5.7	1,440	1,440	100.0	470	470	100.0
L	2,016,000	6,500	0.3	300	270	90.0	74	52	70.3
M	4,500	2,000	44.4	995	95	9.5	234	38	16.2
N	15,000	500	3.3	60	10	16.7	14	0	0.0
O	25,500	2,500	9.8	185	90	48.6	46	2	4.3
P	2,000	0	0.0	35	10	28.6	10	2	20.0
Average	388,162	3,987	......	1,172	1,029	......	530	500	....

A CHECK ON THE ACCURACY OF THE TOTAL COUNTS AS SHOWN IN TABLE 1

A study of table 1 (page 336) shows that the bacteria content of ice cream does not change appreciably during a storage period of reasonable length. In fact, the average counts show as low variation as might reasonably be expected from a number of separate platings from the same sample of almost any dairy product. The question might then arise, do these averages show a normal increase and decrease of the bacteria present in the ice cream, or are they merely coincidences? As a check on this, and also on the method used, the following series of platings were made:

1. Thirteen samples were taken from each of the fourteen cans of ice cream in storage and were plated separately.
2. From one of the thirteen samples from each of the fourteen sets, thirteen weighings and dilutions were made, each being plated separately.
3. From one of the final dilution bottles from each of the fourteen sets, thirteen sets of plates were made.

The results compared to the thirteen averages as shown in table 1, beginning with the frozen ice cream and ending with the sixty-days period

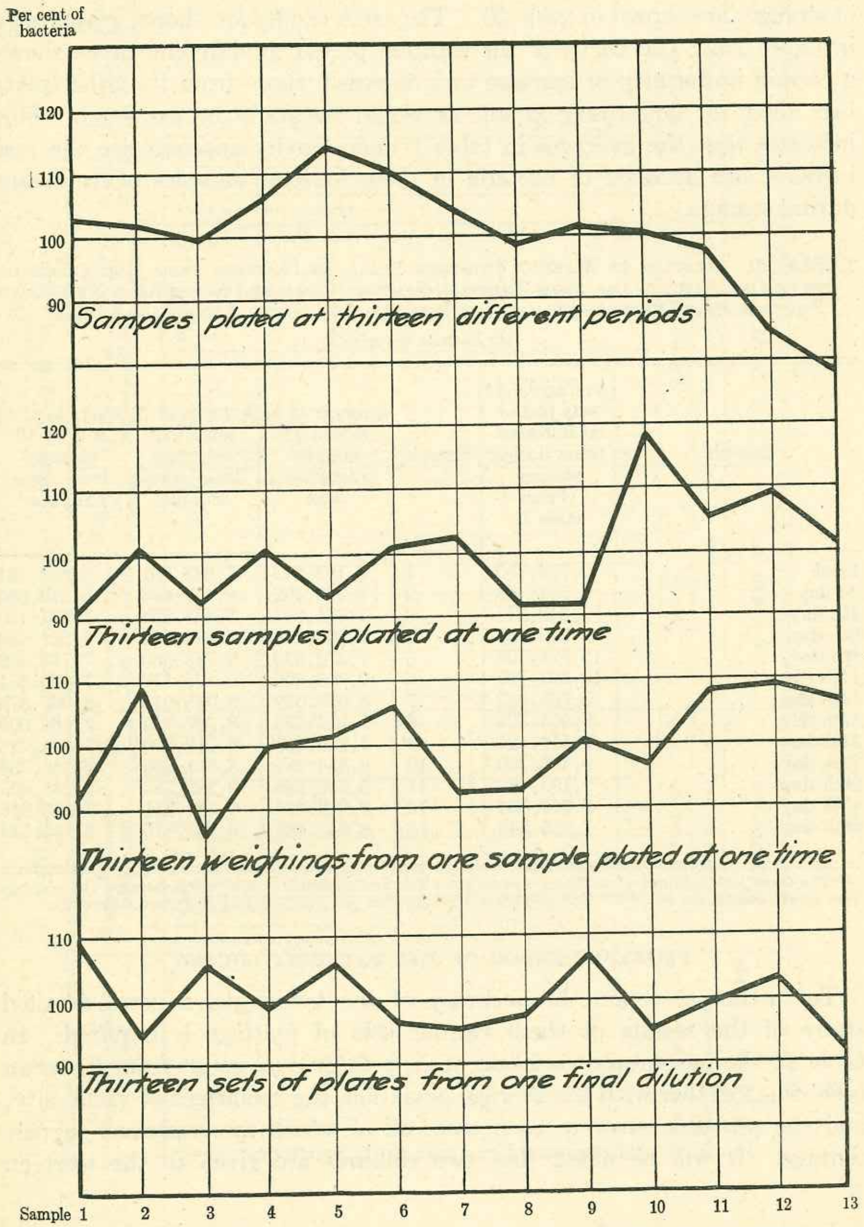

FIG. 76. COMPARATIVE RESULTS FROM DIFFERENT PLATINGS OF ICE CREAM

of storage, are shown in table 20. The same results are shown graphically in figure 76. The curve of the samples plated at different times shows a certain uniformity of increase and decrease; those from the other platings show no uniformity at all, as would naturally be expected. This indicates that the averages in table 1 undoubtedly approximate the real increase and decrease of bacteria in these fourteen samples of ice cream during storage.

TABLE 20. RESULTS OF MAKING THIRTEEN SEPARATE PLATINGS FROM ONE DILUTION, FROM ONE SAMPLE, AND FROM THIRTEEN SAMPLES, COMPARED TO THE SAME NUMBER OF PLATINGS FROM DIFFERENT SAMPLES AT INTERVALS THRUOUT A SIXTY-DAYS PERIOD*

(In bacteria per gram)

Sample	Average of 14 sets plated at different times during storage (From table 1)	Sample	Average of 14 sets of 13 samples plated at once	Average of 14 sets of 13 weighings from same sample	Average of 14 sets of 13 platings from same dilution
Fresh	9,736,750	1	7,182,833	7,937,801	8,003,761
2d day	9,591,428	2	8,093,102	9,450,696	6,919,060
4th day	9,342,871	3	7,325,951	7,435,724	7,717,189
6th day	9,939,982	4	7,966,278	8,654,462	7,251,138
8th day	10,721,707	5	7,372,990	8,778,560	7,718,509
11th day	10,363,707	6	7,992,696	9,161,576	7,070,531
14th day	9,795,532	7	8,099,049	8,018,915	6,967,376
17th day	9,255,325	8	7,273,532	8,027,714	7,164,000
21st day	9,546,421	9	7,250,422	8,718,209	7,824,028
25th day	9,462,750	10	9,354,283	8,371,656	6,959,388
29th day	9,190,482	11	8,352,383	9,363,786	7,287,355
45th day	7,941,564	12	8,649,410	9,451,341	7,553,016
60th day	7,355,549	13	8,043,483	9,252,876	6,754,543

*The three last columns show lower counts than the first column in most cases, because the platings were made during the 45- to 60 days periods of storage, after the numbers had decreased somewhat.

PROBABLE ERROR OF THE BACTERIA COUNTS

To further ascertain the accuracy of the technique, a more detailed study of the results of these various sets of platings is required. In table 21 the percentage variation of each individual count from its mean is shown, together with the average deviation, the coefficient of variability, and the probable error in each case, all of which are expressed in percentage. It will be noted that two columns are given to the thirteen

TABLE 21. VARIABILITY OF INDIVIDUAL COUNTS FROM THE MEAN

Per cent of variation from mean	14 sets of 13 samples plated at once						Number of variates	14 sets of 13 weighings from same sample						Number of variates	14 sets of 13 platings from same dilution						Number of variates	13 sets of 13 platings from same dilution						Number of variates
1	5	5	5	5	5	2	27	5	5	5	5	4	...	24	5	5	5	5	5	2	27	5	5	5	5	5	2	27
3	5	5	5	5	...	...	20	5	5	5	5	2	...	22	5	5	5	5	...	...	20	5	5	5	5	...	...	20
5	5	5	5	5	5	2	27	5	5	5	5	5	...	26	5	5	5	5	5	4	29	5	5	5	5	5	3	28
7	5	5	5	2	...	...	17	5	5	5	2	...	1	17	5	5	5	3	...	...	18	5	5	5	3	...	...	18
9	5	5	3	...	...	...	13	5	5	5	...	...	...	15	5	5	5	1	...	...	16	5	5	5	1	...	...	16
11	5	3	...	...	...	...	8	5	5	1	...	...	...	11	5	5	3	...	...	...	13	5	5	3	...	...	...	13
13	5	5	...	...	...	...	10	5	5	3	...	...	...	13	5	5	...	...	...	...	10	5	5	...	...	...	...	10
15	5	5	3	...	...	...	13	5	5	4	...	...	...	14	5	3	...	...	...	...	8	5	2	...	...	...	...	7
17	5	5	1	...	...	...	11	5	1	...	...	...	...	6	5	5	1	...	...	...	11	5	5	1	...	...	...	11
19	5	3	...	...	...	...	8	5	1	...	...	...	...	6	5	...	...	...	...	...	5	5	...	...	...	...	...	5
21	1	...	...	...	...	...	1	5	...	...	...	...	...	5	4	...	...	...	...	...	4	3	...	...	...	...	...	3
23	5	4	...	...	...	...	9	5	2	...	...	...	...	7	3	...	...	...	...	...	3	2	...	...	...	...	...	2
25	2	...	...	...	...	...	2	3	...	...	...	...	...	3	1	...	...	...	...	...	1	1	...	...	...	...	...	1
27	...	...	...	...	...	...	......	...	...	...	...	...	...	......	2	...	...	...	...	...	2	1	...	...	...	...	...	1
29	1	...	...	...	...	...	1	...	...	...	...	...	...	......	1	...	...	...	...	...	1	1	...	...	...	...	...	1
31	3	...	...	...	...	...	3	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
33	...	...	...	...	...	...	......	1	...	...	...	...	...	1	1	...	...	...	...	...	1	...	...	...	...	...	...	......
35	2	...	...	...	...	...	2	...	...	...	...	...	...	......	1	...	...	...	...	...	1	...	...	...	...	...	...	......
37	...	...	...	...	...	...	......	1	...	...	...	...	...	1	2	...	...	...	...	...	2	1	...	...	...	...	...	1
39	...	...	...	...	...	...	......	1	...	...	...	...	...	1	2	...	...	...	...	...	2	...	...	...	...	...	...	......
41	1	...	...	...	...	...	1	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
43	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
45	1	...	...	...	...	...	1	1	...	...	...	...	...	1	...	...	...	...	...	...	......	...	...	...	...	...	...	......
47	1	...	...	...	...	...	1	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
49	1	...	...	...	...	...	1	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
51	1	...	...	...	...	...	1	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
53	1	...	...	...	...	...	1	...	...	...	...	...	...	......	1	...	...	...	...	...	1	...	...	...	...	...	...	......
55	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......	...	...	...	...	...	...	......
57	...	...	...	...	...	...	......	...	...	...	...	...	...	......	1	...	...	...	...	...	1	...	...	...	...	...	...	......
65	...	...	...	...	...	...	......	...	...	...	...	...	...	......	1	...	...	...	...	...	1	...	...	...	...	...	...	......
Average deviation	11.04 per cent							9.74 per cent							10.36 per cent							8.44 per cent						
Coefficient of variability	15.13 per cent							12.55 per cent							14.60 per cent							10.72 per cent						
Probable error of single variate	±10.21 per cent							± 8.46 per cent							± 9.85 per cent							± 7.23 per cent						

platings from the same dilution bottle, one of fourteen sets and the other of thirteen sets. They are the same except that the latter has one set of platings excluded because of their extreme variation, which did not seem to be at all normal when compared to the other sets. While there was no apparent reason for the extreme variation found in this one set of platings, yet, because of the great difference from any other set, it was felt that the average would be more nearly correct if it were excluded. This is borne out by the fact that when this set is not excluded, the sets showed a greater variability than did those of the thirteen separate weighings, which, because of the additional operations involved, would be expected to show the greater variation.

The comparatively narrow limit of the probable error should be noted. As an illustration, if a can of ice cream having a count of 1,000,000 bacteria per gram is considered, the chances are equal that any one sample from that can would give a count somewhere between 1,102,100 and 897,900. This is comparatively close as bacteria counts go. Or, if several weighed portions for dilution were taken from one sample, it is probable that the count would be within the limits of 1,084,600 and 915,400. And again, if several sets of two plates each were poured from the same final dilution, the probable count of any one set would be between 1,072,300 and 927,700.

In pouring plates from the same final dilution, there was only one mechanical operation to cause variation; while in weighing from the same sample separate portions for dilution and plating, there were from eight to eleven distinct operations to cause variation. This was further increased by one when separate samples were taken from the original package for each plating. Therefore, if the variation in counts was caused solely by the technique used, the probable error should be much smaller than is here shown in the case of platings from the same final dilution, or much larger than is shown in the case of platings from separate weighed portions. There seems, then, to be no other way of explaining the variation in the bacterial counts than to charge the greater part of it to the uncertainty of obtaining a uniform distribution of the bacteria in the final dilution water. Of course every dilution would show more or less error in this respect, but the error would greatly increase with the decrease in the number of bacteria present in a given quantity of water, and therefore it is the final dilution which is the most likely to cause the greatest variation in the count.

In all these comparative platings only two plates were poured in a set, and the counts were calculated from the average of these two. Had more plates been poured and used to determine the counts, it is reasonable to expect that the results would have been more uniform, with a correspondingly lower probable error. To gain an idea of what difference might be expected, the plates in the last series of platings from the final dilution were grouped in sets of four, and the counts, with the probable error of any one count, were calculated on this basis. As before noted, the probable error when sets of two plates each were used was ±7.23 per cent; the probable error when the plates were grouped in sets of four was reduced to ±5.48 per cent.

EFFECT OF DIFFERENT MEDIA ON THE BACTERIA COUNT

Lactose agar and litmus lactose gelatin compared

Thruout these experiments there were one hundred and seventy-seven samples of ice cream plated both on lactose agar and on litmus lactose gelatin. The agar gave the higher count in one hundred and sixty-eight cases, the gelatin in only seven; in two cases the counts were equal. During these platings three different brands of gelatin were used, with practically equal results. The average total count on the agar was 9,193,500 bacteria per gram, while that on the gelatin was 2,773,000, or only 30 per cent of the agar count. This difference was so striking and so uniform that it was thought best to make a series of check platings with plain lactose gelatin, in order to determine whether the difference might be attributed to the use of litmus in the plates.

Litmus lactose gelatin and lactose gelatin compared

The sixty-five samples comparing litmus lactose gelatin and lactose gelatin showed the higher count to result from the plain lactose gelatin in forty-nine cases and from the litmus lactose gelatin in only fourteen; in two cases the counts were equal. The average plate count on the litmus lactose gelatin was 36, while that on the plain lactose gelatin was 47, an increase of 30 per cent. This indicates that the litmus either has an inhibiting action on the growth of the organisms, or that it makes the counting more difficult so that some of the small colonies are not seen and therefore not counted.

The litmus solution used in this work was made as follows: Forty grams of litmus cubes were tied in cheesecloth and allowed to soak for twenty-four hours in 300 cubic centimeters of water. The solution was then filtered into small flasks and sterilized. Two cubic centimeters of this solution was added to each plate when poured.

Lactose agar and lactose gelatin compared

The next series of comparisons was between lactose agar and lactose gelatin, to determine whether or not the gelatin plates would give lower counts than the agar when the litmus was not used. There were fifty-seven comparisons in this series, the average plate counts of the gelatin being 38, while that of the agar was 60. The agar plates showed the higher count in forty-three of these fifty-seven sets. This indicates that the gelatin as a medium was not so efficient as the agar, irrespective of the use of litmus.

Lactose agar, litmus lactose agar, lactose gelatin, and litmus lactose gelatin, compared

In forty-two of the fifty-seven trials just mentioned, litmus lactose agar and litmus lactose gelatin were also used. The average plate counts were as follows:

	Average plate count		
Lactose agar	62	High count	24 times
		Low count	1 time
Litmus lactose agar	51	High count	6 times
		Low count	5 times
Lactose gelatin	44	High count	10 times
		Low count	8 times
Litmus lactose gelatin	28	High count	2 times
		Low count	28 times

This shows that thruout this work the use of gelatin as a medium, and the use of litmus to differentiate between classes of bacteria, both tended to lower the plate count.

SUMMARY

1. An incubation temperature of 20° C. for seven days proved the best of any tried for growing bacteria from the ice cream on agar plates.

2. Of the ingredients used in ice cream, milk, cream, and condensed milk are by far the most prolific sources of bacteria. By effective pasteurization of these products before they enter the mix, ice cream can be made having a low bacteria content.

3. Aside from utensil contamination, there is usually an increase in the number of bacteria, as shown by the plate count in ice cream, resulting from the freezing process. This is probably due to the breaking up of clumps of organisms.

4. There is no radical change in the total number of bacteria in ice cream during storage. There seems, however, to be a tendency toward a slight decrease during the first two to four days, with a more noticeable increase and a corresponding decrease again between the fourth and the twenty-first day, after which time there is a very gradual falling off in numbers.

5. The groups of bacteria in ice cream as determined by litmus gelatin plates and litmus milk tubes do not change noticeably during storage. The acid formers predominate all thru the storage period, and many of them appear to be typical of the *Bacterium lactis acidi* group.

6. The greatest error in making counts of bacteria in ice cream by the plate method seems to be caused by uneven distribution in the final dilution water.

7. Agar plates gave higher counts than did gelatin plates, averaging three times as many bacteria as were found on the litmus gelatin plates. The use of litmus in both the agar and the gelatin plates decreased the counts somewhat.

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